

## **RAW SEQUENCE LISTING**

**The Biotechnology Systems Branch of the Scientific and Technical  
Information Center (STIC) no errors detected.**

Application Serial Number: 10/552,127  
Source: PCT  
Date Processed by STIC: 10/18/2005

# ***ENTERED***

RAW SEQUENCE LISTING                      DATE: 10/18/2005  
PATENT APPLICATION: US/10/552,127        TIME: 10:15:48

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3 <110> APPLICANT: Cirpus, Petra
4 Bauer, Jorg
5 Meyer, Astrid
6 Heinz, Ernst
7 Zahringer, Ulrich
9 <120> TITLE OF INVENTION: DELTA-4 DESATURASES FROM EUGLENA GRACILIS, EXPRESSING
PLANTS, AND
10 OILS CONTAINING PUFA
12 <130> FILE REFERENCE: 12810-00136-US
C--> 14 <140> CURRENT APPLICATION NUMBER: US/10/552,127
C--> 14 <141> CURRENT FILING DATE: 2005-10-05
14 <150> PRIOR APPLICATION NUMBER: PCT/EP2004/003628
15 <151> PRIOR FILING DATE: 2004-04-06
17 <150> PRIOR APPLICATION NUMBER: DE 103 16 267.4
18 <151> PRIOR FILING DATE: 2003-04-08
20 <160> NUMBER OF SEQ ID NOS: 7
22 <170> SOFTWARE: PatentIn version 3.3
24 <210> SEQ ID NO: 1
25 <211> LENGTH: 1626
26 <212> TYPE: DNA
27 <213> ORGANISM: Euglena gracilis
29 <220> FEATURE:
30 <221> NAME/KEY: CDS
31 <222> LOCATION: (1)..(1623)
32 <223> OTHER INFORMATION: Delta-4-Desaturase
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35 atg ttg gtg ctg ttt ggc aat ttc tat gtc aag caa tac tcc caa aag 48
36 Met Leu Val Leu Phe Gly Asn Phe Tyr Val Lys Gln Tyr Ser Gln Lys
37 1 5 10 15
39 aac ggc aag ccg gag aac gga gcc acc cct gag aac gga gcg aag ccg 96
40 Asn Gly Lys Pro Glu Asn Gly Ala Thr Pro Glu Asn Gly Ala Lys Pro
41 20 25 30
43 caa cct tgc gag aac ggc acg gtg gaa aag cga gag aat gac acc gcc 144
44 Gln Pro Cys Glu Asn Gly Thr Val Glu Lys Arg Glu Asn Asp Thr Ala
45 35 40 45
47 aac gtt cgg ccc acc cgt cca gct gga ccc ccg ccg gcc acg tac tac 192
48 Asn Val Arg Pro Thr Arg Pro Ala Gly Pro Pro Pro Ala Thr Tyr Tyr
49 50 55 60
51 gac tcc ctg gca gtg tcg ggg cag ggc aag gag cgg ctg ttc acc acc 240
52 Asp Ser Leu Ala Val Ser Gly Gln Gly Lys Glu Arg Leu Phe Thr Thr
53 65 70 75 80
55 gat gag gtg agg cgg cac atc ctc ccc acc gat ggc tgg ctg acg tgc 288
56 Asp Glu Val Arg Arg His Ile Leu Pro Thr Asp Gly Trp Leu Thr Cys
57 85 90 95

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Input Set : A:\Sequence listing-12810-00136-US.txt

Output Set: N:\CRF4\10182005\J552127.raw

59	cac gaa gga gtc tac gat gtc act gat ttc ctt gcc aag cac cct ggt	336
60	His Glu Gly Val Tyr Asp Val Thr Asp Phe Leu Ala Lys His Pro Gly	
61	100 105 110	
63	ggc ggt gtc atc acg ctg ggc ctt gga agg gac tgc aca atc ctc atc	384
64	Gly Gly Val Ile Thr Leu Gly Leu Gly Arg Asp Cys Thr Ile Leu Ile	
65	115 120 125	
67	gag tca tac cac cct gct ggg cgc ccg gac aag gtg atg gag aag tac	432
68	Glu Ser Tyr His Pro Ala Gly Arg Pro Asp Lys Val Met Glu Lys Tyr	
69	130 135 140	
71	cgc att ggt acg ctg cag gac ccc aag acg ttc tat gct tgg gga gag	480
72	Arg Ile Gly Thr Leu Gln Asp Pro Lys Thr Phe Tyr Ala Trp Gly Glu	
73	145 150 155 160	
75	tcc gat ttc tac cct gag ttg aag cgc cgg gcc ctt gca agg ctg aag	528
76	Ser Asp Phe Tyr Pro Glu Leu Lys Arg Arg Ala Leu Ala Arg Leu Lys	
77	165 170 175	
79	gag gct ggt cag gcg cgg cgc ggc ggc ctt ggg gtg aag gcc ctc ctg	576
80	Glu Ala Gly Gln Ala Arg Arg Gly Gly Leu Gly Val Lys Ala Leu Leu	
81	180 185 190	
83	gtg ctc acc ctc ttc ttc gtg tcg tgg tac atg tgg gtg gcc cac aag	624
84	Val Leu Thr Leu Phe Phe Val Ser Trp Tyr Met Trp Val Ala His Lys	
85	195 200 205	
87	tcc ttc ctc tgg gcc gcc gtc tgg ggc ttc gcc ggc tcc cac gtc ggg	672
88	Ser Phe Leu Trp Ala Ala Val Trp Gly Phe Ala Gly Ser His Val Gly	
89	210 215 220	
91	ctg agc atc cag cac gat ggc aac cac ggc gcg ttc agc cgc aac aca	720
92	Leu Ser Ile Gln His Asp Gly Asn His Gly Ala Phe Ser Arg Asn Thr	
93	225 230 235 240	
95	ctg gtg aac cgc ctg gcg ggg tgg ggc atg gac ttg atc ggc gcg tcg	768
96	Leu Val Asn Arg Leu Ala Gly Trp Gly Met Asp Leu Ile Gly Ala Ser	
97	245 250 255	
99	tcc acg gtg tgg gag tac cag cac gtc atc ggc cac cac cag tac acc	816
100	Ser Thr Val Trp Glu Tyr Gln His Val Ile Gly His His Gln Tyr Thr	
101	260 265 270	
103	aac ctc gtg tcg gac acg cta ttc agt ctg cct gag aac gat ccg gac	864
104	Asn Leu Val Ser Asp Thr Leu Phe Ser Leu Pro Glu Asn Asp Pro Asp	
105	275 280 285	
107	gtc ttc tcc agc tac ccg ctg atg cgc atg cac ccg gat acg gcg tgg	912
108	Val Phe Ser Ser Tyr Pro Leu Met Arg Met His Pro Asp Thr Ala Trp	
109	290 295 300	
111	cag ccg cac cac cgc ttc cag cac ctg ttc gcg ttc cca ctg ttc gcc	960
112	Gln Pro His His Arg Phe Gln His Leu Phe Ala Phe Pro Leu Phe Ala	
113	305 310 315 320	
115	ctg atg aca atc agc aag gtg ctg acc agc gat ttc gct gtc tgc ctc	1008
116	Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu	
117	325 330 335	
119	agc atg aag aag ggg tcc atc gac tgc tcc tcc agg ctc gtc cca ctg	1056
120	Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu	
121	340 345 350	
123	gag ggg cag ctg ctg ttc tgg ggg gcc aag ctg gcg aac ttc ctg ttg	1104

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124 Glu Gly Gln Leu Leu Phe Trp Gly Ala Lys Leu Ala Asn Phe Leu Leu
125          355          360          365
127 cag att gtg ttg cca tgc tac ctc cac ggg aca gct atg ggc ctg gcc      1152
128 Gln Ile Val Leu Pro Cys Tyr Leu His Gly Thr Ala Met Gly Leu Ala
129          370          375          380
131 ctc ttc tct gtt gct cac ctt gtg tgc ggg gag tac ctc gcg atc tgc      1200
132 Leu Phe Ser Val Ala His Leu Val Ser Gly Glu Tyr Leu Ala Ile Cys
133 385          390          395          400
135 ttc atc atc aac cac atc agc gag tct tgt gag ttt atg aat aca agc      1248
136 Phe Ile Ile Asn His Ile Ser Glu Ser Cys Glu Phe Met Asn Thr Ser
137          405          410          415
139 ttt caa acc gcc gcc cgg agg aca gag atg ctt cag gca gca cat cag      1296
140 Phe Gln Thr Ala Ala Arg Arg Thr Glu Met Leu Gln Ala Ala His Gln
141          420          425          430
143 gca gcg gag gcc aag aag gtg aag ccc acc cct cca ccg aac gat tgg      1344
144 Ala Ala Glu Ala Lys Lys Val Lys Pro Thr Pro Pro Asn Asp Trp
145          435          440          445
147 gct gtg aca cag gtc caa tgc tgc gtg aat tgg aga tca ggt ggc gtg      1392
148 Ala Val Thr Gln Val Gln Cys Cys Val Asn Trp Arg Ser Gly Gly Val
149          450          455          460
151 ttg gcc aat cac ctc tct gga ggc ttg aac cac cag atc gag cat cat      1440
152 Leu Ala Asn His Leu Ser Gly Gly Leu Asn His Gln Ile Glu His His
153 465          470          475          480
155 ctg ttc ccc agc atc tgc cat gcc aac tac ccc acc atc gcc cct gtt      1488
156 Leu Phe Pro Ser Ile Ser His Ala Asn Tyr Pro Thr Ile Ala Pro Val
157          485          490          495
159 gtg aag gag gtg tgc gag gag tac ggg ttg ccg tac aag aat tac gtc      1536
160 Val Lys Glu Val Cys Glu Glu Tyr Gly Leu Pro Tyr Lys Asn Tyr Val
161          500          505          510
163 acg ttc tgg gat gca gtc tgt ggc atg gtt cag cac ctc cgg ttg atg      1584
164 Thr Phe Trp Asp Ala Val Cys Gly Met Val Gln His Leu Arg Leu Met
165          515          520          525
167 ggt gct cca ccg gtg cca acg aac ggg gac aaa aag tca taa      1626
168 Gly Ala Pro Pro Val Pro Thr Asn Gly Asp Lys Lys Ser
169          530          535          540
172 <210> SEQ ID NO: 2
173 <211> LENGTH: 541
174 <212> TYPE: PRT
175 <213> ORGANISM: Euglena gracilis
177 <400> SEQUENCE: 2
179 Met Leu Val Leu Phe Gly Asn Phe Tyr Val Lys Gln Tyr Ser Gln Lys
180 1          5          10          15
183 Asn Gly Lys Pro Glu Asn Gly Ala Thr Pro Glu Asn Gly Ala Lys Pro
184          20          25          30
187 Gln Pro Cys Glu Asn Gly Thr Val Glu Lys Arg Glu Asn Asp Thr Ala
188          35          40          45
191 Asn Val Arg Pro Thr Arg Pro Ala Gly Pro Pro Pro Ala Thr Tyr Tyr
192          50          55          60
195 Asp Ser Leu Ala Val Ser Gly Gln Gly Lys Glu Arg Leu Phe Thr Thr

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196 65          70          75          80
199 Asp Glu Val Arg Arg His Ile Leu Pro Thr Asp Gly Trp Leu Thr Cys
200          85          90          95
203 His Glu Gly Val Tyr Asp Val Thr Asp Phe Leu Ala Lys His Pro Gly
204          100          105          110
207 Gly Gly Val Ile Thr Leu Gly Leu Gly Arg Asp Cys Thr Ile Leu Ile
208          115          120          125
211 Glu Ser Tyr His Pro Ala Gly Arg Pro Asp Lys Val Met Glu Lys Tyr
212          130          135          140
215 Arg Ile Gly Thr Leu Gln Asp Pro Lys Thr Phe Tyr Ala Trp Gly Glu
216 145          150          155          160
219 Ser Asp Phe Tyr Pro Glu Leu Lys Arg Arg Ala Leu Ala Arg Leu Lys
220          165          170          175
223 Glu Ala Gly Gln Ala Arg Arg Gly Gly Leu Gly Val Lys Ala Leu Leu
224          180          185          190
227 Val Leu Thr Leu Phe Phe Val Ser Trp Tyr Met Trp Val Ala His Lys
228          195          200          205
231 Ser Phe Leu Trp Ala Ala Val Trp Gly Phe Ala Gly Ser His Val Gly
232          210          215          220
235 Leu Ser Ile Gln His Asp Gly Asn His Gly Ala Phe Ser Arg Asn Thr
236 225          230          235          240
239 Leu Val Asn Arg Leu Ala Gly Trp Gly Met Asp Leu Ile Gly Ala Ser
240          245          250          255
243 Ser Thr Val Trp Glu Tyr Gln His Val Ile Gly His His Gln Tyr Thr
244          260          265          270
247 Asn Leu Val Ser Asp Thr Leu Phe Ser Leu Pro Glu Asn Asp Pro Asp
248          275          280          285
251 Val Phe Ser Ser Tyr Pro Leu Met Arg Met His Pro Asp Thr Ala Trp
252          290          295          300
255 Gln Pro His His Arg Phe Gln His Leu Phe Ala Phe Pro Leu Phe Ala
256 305          310          315          320
259 Leu Met Thr Ile Ser Lys Val Leu Thr Ser Asp Phe Ala Val Cys Leu
260          325          330          335
263 Ser Met Lys Lys Gly Ser Ile Asp Cys Ser Ser Arg Leu Val Pro Leu
264          340          345          350
267 Glu Gly Gln Leu Leu Phe Trp Gly Ala Lys Leu Ala Asn Phe Leu Leu
268          355          360          365
271 Gln Ile Val Leu Pro Cys Tyr Leu His Gly Thr Ala Met Gly Leu Ala
272          370          375          380
275 Leu Phe Ser Val Ala His Leu Val Ser Gly Glu Tyr Leu Ala Ile Cys
276 385          390          395          400
279 Phe Ile Ile Asn His Ile Ser Glu Ser Cys Glu Phe Met Asn Thr Ser
280          405          410          415
283 Phe Gln Thr Ala Ala Arg Arg Thr Glu Met Leu Gln Ala Ala His Gln
284          420          425          430
287 Ala Ala Glu Ala Lys Lys Val Lys Pro Thr Pro Pro Pro Asn Asp Trp
288          435          440          445
291 Ala Val Thr Gln Val Gln Cys Val Asn Trp Arg Ser Gly Gly Val
292          450          455          460

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```

295 Leu Ala Asn His Leu Ser Gly Gly Leu Asn His Gln Ile Glu His His
296 465                               470                               475                               480
299 Leu Phe Pro Ser Ile Ser His Ala Asn Tyr Pro Thr Ile Ala Pro Val
300                               485                               490                               495
303 Val Lys Glu Val Cys Glu Glu Tyr Gly Leu Pro Tyr Lys Asn Tyr Val
304                               500                               505                               510
307 Thr Phe Trp Asp Ala Val Cys Gly Met Val Gln His Leu Arg Leu Met
308                               515                               520                               525
311 Gly Ala Pro Pro Val Pro Thr Asn Gly Asp Lys Lys Ser
312 530                               535                               540

```

315 &lt;210&gt; SEQ ID NO: 3

316 &lt;211&gt; LENGTH: 26

317 &lt;212&gt; TYPE: DNA

318 &lt;213&gt; ORGANISM: Unknown

320 &lt;220&gt; FEATURE:

321 &lt;221&gt; NAME/KEY: misc\_feature

322 &lt;222&gt; LOCATION: (1)..(26)

NO: 323 <223> OTHER INFORMATION: Forward primer for cloning the shorter reading frame (SEQ ID

324 1) into the vector pYES2

326 &lt;400&gt; SEQUENCE: 3

327 ggtaccatgt tgggtgctggt tggcaa 26

330 &lt;210&gt; SEQ ID NO: 4

331 &lt;211&gt; LENGTH: 25

332 &lt;212&gt; TYPE: DNA

333 &lt;213&gt; ORGANISM: Unknown

335 &lt;220&gt; FEATURE:

336 &lt;221&gt; NAME/KEY: misc\_feature

337 &lt;222&gt; LOCATION: (1)..(25)

NO: 338 <223> OTHER INFORMATION: Reverse primer for cloning the shorter reading frame (SEQ ID

339 1) into the vector pYES2

341 &lt;400&gt; SEQUENCE: 4

342 ctcgagttat gactttttgt ccccg 25

345 &lt;210&gt; SEQ ID NO: 5

346 &lt;211&gt; LENGTH: 28

347 &lt;212&gt; TYPE: DNA

348 &lt;213&gt; ORGANISM: Unknown

350 &lt;220&gt; FEATURE:

351 &lt;221&gt; NAME/KEY: misc\_feature

352 &lt;222&gt; LOCATION: (1)..(28)

end 353 <223> OTHER INFORMATION: Forward primer for introducing NotI cleavage sites at the 5'

354 of the coding sequence

356 &lt;400&gt; SEQUENCE: 5

357 gcggccgcat gttggtgctg tttggcaa 28

360 &lt;210&gt; SEQ ID NO: 6

361 &lt;211&gt; LENGTH: 25

362 &lt;212&gt; TYPE: DNA

363 &lt;213&gt; ORGANISM: Unknown

365 &lt;220&gt; FEATURE:

366 &lt;221&gt; NAME/KEY: misc\_feature

**VERIFICATION SUMMARY**

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Input Set : A:\Sequence listing-12810-00136-US.txt

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L:14 M:270 C: Current Application Number differs, Replaced Current Application No

L:14 M:271 C: Current Filing Date differs, Replaced Current Filing Date